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Melatonin ameliorates oxidative damage induced by maternal lead exposure in rat pups

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HIGHLIGHTS

- Pb induce oxidative stress and change antioxidant enzyme activities in cerebellum.
- Melatonin administration reduce lead-induced oxidative stress in cerebellum.
- Melatonin administration recover lead-induced motor behavior deficits.

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ABSTRACT

During the particular period of cerebellum development, exposure to lead (Pb) decreases cerebellum growth and can result in selective loss of neurons. The detection and prevention of Pb toxicity is a major international public health priorities. This research study was conducted to evaluate the effects of melatonin, an effective antioxidant and free radical scavenger, on Pb induced neurotoxicity and oxidative stress in the cerebellum. Pb exposure was initiated on gestation day 5 with the addition of daily doses of 0.2% lead acetate to distilled drinking water and continues until weaning. Melatonin (10 mg/kg) was given once daily at the same time. 21 days after birth, several antioxidant enzyme activities including superoxide dismutase (SOD) and glutathione peroxidase (GPx) were assayed. Thiobarbituric acid reactive substance (TBARS) levels were measured as a marker of lipid peroxidation. Rotarod and locomotor activity tests were performed on postnatal days (PDs) 31–33 and a histological study was performed after completion of behavioral measurements on PD 33. The results of the present work demonstrated that Pb could induce lipid peroxidation, increase TBARS levels and decrease GPx and SOD activities in the rat cerebellum. We also observed that Pb impaired performance on the rotarod and locomotor activities of rats. However, treatment with melatonin significantly attenuated the motoric impairment and lipid peroxidation process and restored the levels of antioxidants. Histological analysis indicated that Pb could decrease Purkinje cell count and melatonin prevented this toxic effect. These results suggest that treatment with melatonin can improve motor deficits and oxidative stress by protecting the cerebellum against Pb toxicity.

1. Introduction

Pb is a heavy metal with no apparent biological function. The widespread environmental contamination, the propensity to cause a wide spectrum of toxic effects and the number of individuals affected worldwide make this ubiquitous neurotoxicant a public health problem of global magnitude [1]. Unfortunately, Pb poisoning cases continue to occur in many countries. Today, it is used in many products including batteries, solder, paint, fishing weights, pottery glaze and ammunition. Pb has also been added to gasoline to reduce engine knocking. It can also be found in some imported cosmetics. Pb contaminating drinking water is most often a problem in houses that are either very old or very new. The source of Pb in such water is most likely plumbing pipes or solders. Pb exposure occurs mainly via eating, drinking or inhalation and its deposition is evident in several tissues, such as the kidney, liver, brain and bones [2,3]. The nervous system is the primary target for the low levels of Pb exposure and the developing brain appears to be especially vulnerable to Pb neurotoxicity [4–7] while the cerebellum has been described as a favorable target for Pb pathology [8,9]. In this regard, cerebellar damage has been pointed as a crucial event related to Pb-induced motor deficits [10].

The cerebellum involves in movement coordination and maintenance of balance. It co-ordinates the different muscle groups so that the muscle exerts movements fluently and precisely. The cerebellum receives continual feedback information about intended movement

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and actual movements. Purkinje neurons, which are thought to be the sole output of the cerebellar cortex, encode the timing signal required for motor behavior in their firing precision and pattern activity. The Purkinje cells and the granule cells are the most important targets in the cerebellum for toxic substances. Atrophy of the cerebellum is usually accompanied by different forms of ataxia and an unstable gait [11].

Environmental exposure to Pb produces behavioral, physiological and biochemical deficiencies in humans [12]. Pb, a systemic toxicant affecting virtually every organ system, primarily affects the central nervous system, particularly the developing brain. Consequently, children are at a greater risk of suffering from the neurotoxic effects of Pb than adults. The ability of Pb to pass through the blood–brain barrier is due in large part to its ability to substitute for calcium ions. Within the brain, Pb-induced damage in the prefrontal cerebral cortex, hippocampus, and cerebellum can lead to a variety of neurological disorders, such as brain damage, mental retardation, behavioral problems, nerve damage, and possibly Alzheimer's disease, Parkinson's disease, and schizophrenia [13].

Pb exposure has been shown to cause generation of excessive amounts of reactive oxygen species (ROS) and alteration of antioxidant defense systems in animals [14,15] and in occupationally exposed workers [16]. Therefore, it has been suggested that Pb-induced oxidative stress contributes to the pathogenesis of Pb poisoning by disrupting the pro-/antioxidant balance in the cells [17].

It is also known that Pb poisoning exerts its most severe consequences in the developing brain due to the immature blood–brain barrier and the absence of protein complexes able to sequester Pb in mature tissue [18], along with the intense cellular proliferation, differentiation and synaptogenesis that occur in this period. Pb is absorbed through the placenta during pregnancy and passes into milk during the lactation period, reaching the developing brain where it preferentially impacts the functionality and morphology of the hippocampus [19,20]. Although the Pb-induced impairment in cognitive and motor functions is extensively studied, very few studies have looked into the possible ways of preventing this Pb-induced deficit.

Antioxidants with strong chelating potential play a major role in the treatment of Pb poisoning because oxidative stress, induced by the presence of Pb in several organs, appears to be a component of the molecular mechanism of Pb toxicity. Induction of harmful free radicals by Pb, and subsequent depletion of a cell's antioxidant defenses, can result in generalized disruption of the prooxidant/antioxidant balance in Pb-burdened tissues and contribute to tissue injury via oxidative damage to critical biomolecules [21].

Melatonin, a hormone produced by the pineal gland, has many physiological functions, including chronomodulation of biological systems and regulation of immune functions, acting as either an inhibitor or activator of immune responses [22]. Moreover, melatonin prevents oxidative stress both through its free radical scavenging effect and by directly increasing antioxidant activity [23], so different studies have demonstrated its protective role against oxidative damage induced by drugs and toxins [24]. It is well known that one of the earliest pathological events secondary to oxygen free radical injury is oxidative damage and the generation of ROS caused by oxidative stress, which have an important role in provoking neural death [25]. The developing embryo and fetus are substantially deficient in most antioxidative enzymes, and may be at high risk from the embryopathic effects of both endogenous and exogenously enhanced oxidative stress and ROS [26]. Melatonin is rapidly transferred from maternal to fetal circulation and brain tissue [27]. Also, melatonin prevents oxidative damage of lipids and DNA and mitochondrial damage in the mature fetal rat brain [26].

Melatonin protects neuroblastoma cells against Pb-induced apoptosis [28], and prevents oxidative DNA damage in blood lymphocytes in vitro by scavenging reactive oxygen species [29]. Despite numerous reports of the beneficial effects of melatonin against Pb toxicity, little is known about the influence of melatonin on pre- and neonatal lead exposure in the cerebellum. Therefore, the aim of this study was to examine the protective effects of melatonin on Pb-induced neurotoxicity and oxidative stress in the developing rat cerebellum.

2. Methodology

2.1. Drugs and chemicals

Lead acetate, melatonin, 2-thiobarbituric acid (TBA), 1.1.3.3tetramethoxypropa, nitro blue tetrazolium (NBT), and trichloroacetic acid (TCA), were all purchased from Sigma-Aldrich chemicals.

2.2. Experimental animals and treatment

The experimental protocol was approved by the Research and Ethics Committee of Damghan University. Adult female and male Wistar rats were obtained from the breeding colony of the Pasture Institute of Iran. They were housed in a temperature and light controlled room under a 12/12 h light/dark cycle with food and water provided ad libitum. After one week acclimatization in the laboratory conditions, female rats were housed overnight with males and checked on the following morning for the presence of copulation plugs. The day at which a vaginal plug found was used to define the beginning of gestation (day 0). Pregnant females were individually housed in plastic cages. Pregnant rats were randomly divided into five different treatment groups of 5 rats: control, melatonin (Mel), lead (Pb), lead + vehicle (Pb + veh), and lead + melatonin (Pb + Mel) groups. Mothers of the control group were given distilled drinking water. Mothers of the melatonin group were given distilled drinking water and melatonin (10 mg/kg) once daily through oral gavage at 16:00 h. Mothers of the Pb group were given daily doses of 0.2% lead acetate dissolved in distilled drinking water. This solution was made daily to prevent the precipitation of Pb as lead carbonate. Mothers of the Pb + veh group were given daily doses of 0.2% lead acetate dissolved in distilled drinking water and melatonin solvent (2% alcohol in saline) by oral gavage once daily at 16:00 h. In order to study the protective effect of melatonin, another group of mothers was administered with melatonin (10 mg/kg) through oral gavage at 16:00 h in addition to exposure to lead acetate (0.2%).

All groups were treated from the 5th day of gestation until weaning (PD 21). The lead acetate dosage was selected on the basis of earlier reports which have demonstrated its neurotoxin effects in rat pups [30–32]. The melatonin dosage was selected on the basis of earlier reports which have demonstrated its neuroprotective effects in rats [25,33]. Melatonin was dissolved in ethanol and further diluted in saline. The final concentration of alcohol was <2%. At birth, eight pups were left with each dam. Whenever possible, only male rats were kept within the litters and females were kept only if necessary to maintain equal litter sizes. Pups were not directly exposed to Pb. At postnatal day 21, 5–8 pups (per group) were used to evaluate cerebellar Pb concentrations, TBARS levels, and SOD and GPx activities and the other 10 pups (per group) were used for behavioral and histological evaluation on PDs 31 and 33, respectively. The design of the experiment is shown in Fig. 1.

2.3. Cerebellar Pb concentration

At the end of the treatment (on PD 21), pups were sacrificed by decapitation under ketamine–xylazine anesthesia and cerebellums were removed. For cerebellar metal determination, wet tissue weight was recorded. After digestion with concentrated nitric acid using a microwave digestion system (model MDS-2100, CEM, USA), samples were brought to a constant volume and determination of tissue Pb contents was performed using an atomic absorption spectrophotometer (AAS, Perkin Elmer model AAnalyst 100) [34].